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Research Progress on The Role of Splicing Factors in Hepatocellular Carcinoma

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Abstract

Splicing factors are proteins that mediate the RNA splicing process, playing a significant role in tumor metastasis by regulating molecular mechanisms and selective splicing patterns of RNA. They are closely associated with the occurrence and development of hepatocellular carcinoma, promoting tumor formation through various mechanisms such as cell proliferation, apoptosis, migration, enhanced metastatic potential, treatment resistance, and immune evasion. Splicing factors also play a crucial role in the diagnosis, treatment, and prognosis of hepatocellular carcinoma. Despite considerable advancements in splicing factor research in hepatocellular carcinoma, the precise mechanisms by which they are utilized remain unknown. Further investigation is necessary to elucidate their precise contribution to the pathogenesis of hepatocellular carcinoma.

Key words: Splicing Factors; Hepatocellular Carcinoma; Tumor metastasis; Treatment resistance; Immune evasion

BACKGROUND INTRODUCTION

RNA splicing is the process of eliminating introns from the precursor mRNA molecule and joining exons together to create mature mRNA. The process is facilitated by a central spliceosome composed of five short nuclear RNAs (including U1, U2, U4, U5, and U6) and several splicing-related proteins categorized as splice factors (SFs) (1). The recognized SF family includes two types: serine/arginine-rich proteins (2) and classical hnRNP proteins (3).

The worldwide incidence of liver cancer in 2020 was 905,677 cases, resulting in 830,180 deaths. The predominant kind of liver cancer was hepatocellular carcinoma (HCC) (4-5). HCC has a significant occurrence and death rate, an intricate development process, several risk factors, and is distinguished by late detection, limited chances for curative surgery, and an unfavorable prognosis. The cumulative survival percentage for patients over a period of 5 years is around 21% (6).

There is mounting evidence linking alterations in SFs expression to tumor development, cancer metastasis, and treatment resistance. Multiple malignancies have been shown to have elevated SF2/ASF, which controls the variable splicing of the tumor suppressor BIN1 isoform, therefore inhibiting the tumor suppressor function of the BIN1 isoform (7). In addition, (8) discovered that the upregulation of SRp20 (SRSF3) enhances tumor formation in nude mice and supports tumor progression. Glioblastoma exhibits increased levels of hnRNP A2/B1, which is associated with a negative prognosis. Conversely, reduced expression of hnRNP A2/B1 suppresses tumor development in glioblastoma cells (9).

Although a comprehensive investigation of spliceosome gene expression at the protein level in cancer has not been documented, several studies have shown the upregulation of certain spliceosome genes. Previous research has demonstrated increased expression of spliceosome genes HSPA1A, SNRPE, TRA2B, and PRPF19 in cancerous tissues at the protein level(10-18). Reports suggest that U1 small nuclear ribonucleoproteins (snRNP), necessary for the creation of spliceosomes, might hinder the movement and infiltration of different kinds of cancer cells(19). SF3B4, an essential constituent of the U2 pre-mRNA spliceosome complex, has recently been identified as a possible oncogene in HCC(20).

VARIABLE SPLICING IN TUMOR METASTASIS

SF proteins often control the process of RNA splicing by identifying certain genetic elements inside alternative exons or neighboring introns (3). RNA splicing dysregulation is a prevalent molecular characteristic seen in almost all forms of malignancies. The occurrence of splicing modifications in cancer is mostly attributed to the frequent mutations and alterations in the expression of trans-acting factors that govern the process of splicing catalysis and regulation (21).

MOLECULAR MECHANISM OF RNA SPLICING

The process of producing mature mRNA by removing intron sequences from precursor-mRNA is an indispensable procedure that regulates the expression of over 95% of human genes. The process of precursor-mRNA splicing is crucial to the diversity of proteins. The process of splicing is predominantly dependent on auxiliary proteins and snRNP. Humans possess two distinct varieties of introns: the uncommon U12 type intron and the common U2 type intron, which account for approximately 99 percent. Separate SFs (U2 spliceosome and U12 spliceosome, respectively) eliminate these two varieties of introns, which have distinct splicing consensus sequences. Different base sequences, including splice donor sites (5′ end), branch sites (near the 3′ end), and acceptor sites (3′ end of the

intron), influence the assembly of SFs with precursor-mRNA. The branch site of the U2-type spliceosome bonds with U2 snRNP, whereas the supply site primarily binds with U1 snRNP. Multiple SFs interact early in the process of spliceosome formation and intron recognition to produce U1 and U2 snRNP (22).

RNA VARIABLE SPLICING MODE

Research has indicated that the occurrence and development of tumor cells involve diverse forms of alternative splicing (AS). These modes primarily consist of four types: exon skipping, selective 5′ splicing, selective 3′ splicing, and intron retention (Fig. 1). "Exon skipping" represents the prevailing form of AS observed in both invertebrates and vertebrates (23).

SFS AND THE OCCURRENCE AND DEVELOPMENT OF HCC

Cancer-associated aberrations in splicing regulation may facilitate the development of tumors via many pathways, resulting in heightened cellular proliferation, reduced cell death, augmented migratory and metastatic capabilities, resistance to chemotherapy, and evasion of immunological detection (21). SFs, which are crucial regulators of post-transcriptional gene expression, significantly contribute to the onset and progression of cancer (24). As shown in Fig. 2. Somatic mutations or aberrant activation of transcription factors or signaling pathways can result in mutations or alterations in the expression of SFs in cancer. This, in turn, leads to corresponding splicing events, generating diverse protein subtypes associated with cancer. These subtypes play a role in regulating the proliferation, apoptosis, invasion, and metabolism of cancer cells, ultimately inducing the manifestation of aggressive malignant characteristics. Aberrant expression of SFs in cancer may upregulate the expression of anti-apoptotic genes including BCL2L1, CASP2, and FAS, as well as promote the splicing of CD44, FGFR2, RAC1, and RMST1R into cancer-promoting subtypes that facilitate cancer cell invasion (25).

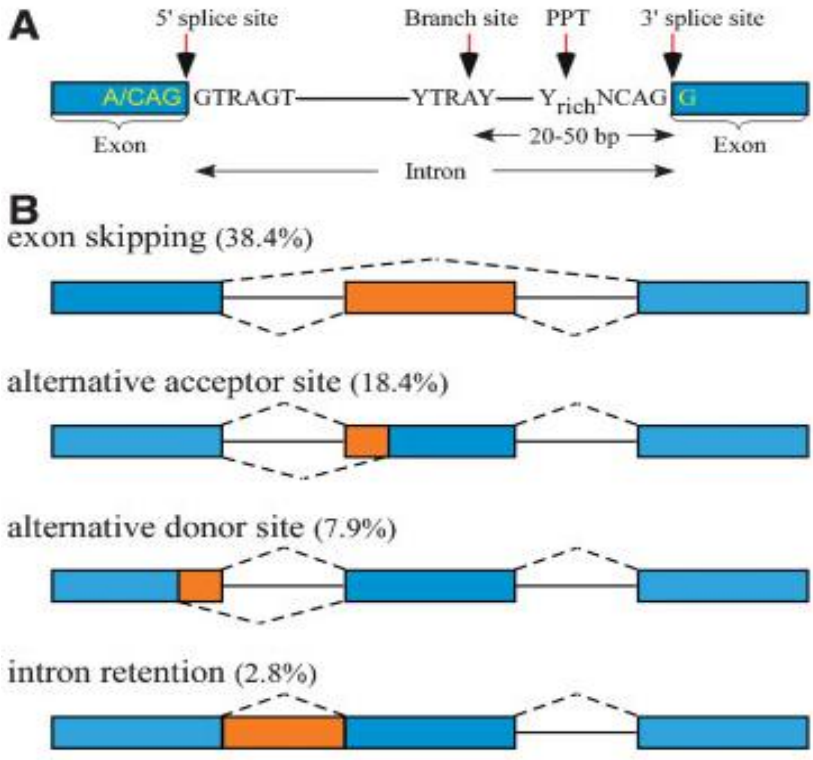

Figure 1. Four common forms of RNA alternative splicing (23)

In terms of clinical relevance, HSPB1, DDX39A, and NELFE are considered to be three of the most significant SFs. HSPB1, commonly referred to as Hsp27, is upregulated in several cancer types (26). Prior research has shown that Hsp27 is increased in HCC and contributes to the invasiveness that promotes the malignancy of HCC (27-29). Similarly, the expression of DDX39A is increased in HCC, and it enhances the development and spread of HCC (30-31).

The expression of SF3B1 is elevated and is correlated with the invasiveness of tumors as well as the expression of oncogenic splicing variants (KLF6-SV1, BCL-XL) (32). The RNA-binding protein SF3B4 regulates the enabled homolog, which intensifies the proliferation, invasion, and migration of liver cancer cells in HCC via the Notch signaling pathway (33). The small nuclear ribonucleoprotein polypeptide A (SNRPA) is linked to MVI in HCC. SNRPA is highly expressed in MVI-HCC and is associated with unfavourable patient survival. SNRPA enhances the epithelial-mesenchymal transition (EMT) process in HCC cells by activating the NOTCH1/Snail pathway in both laboratory and living organism settings, hence hastening metastasis (34).

MicroRNAs participate in the control of gene expression after the process of transcription. They specifically attach to the 3' untranslated region (3'-UTR) of their target mRNAs in order to inhibit their expression (35). Mice with a deficiency of SRSF3 in liver cells are more likely to develop HCC. Research has shown that SRSF3 has a significant role in the prevention of HCC by controlling the process of splicing to hinder fibrosis, mitotic splicing, and EMT (36).

In HCC in humans, the levels of SFs, such as the tumor suppressor SRSF3, and the regulation of AS in the genes examined, are often increased in tumor tissues compared to non-tumor tissues (37). Inhibiting the migration and proliferation of HCC cells is possible by downregulating the expression of SMNDC1, which is overexpressed in tumor cells (38). As an important SF, SRSF9 controls cell migration and proliferation, which in turn influences the Wnt and cell cycle pathways, and thus impacts the cancerous development of HCC (39). A poor prognosis is associated with increased SRSF9 expression because it is significantly connected with the malignant pathophysiological features of HCC (40).

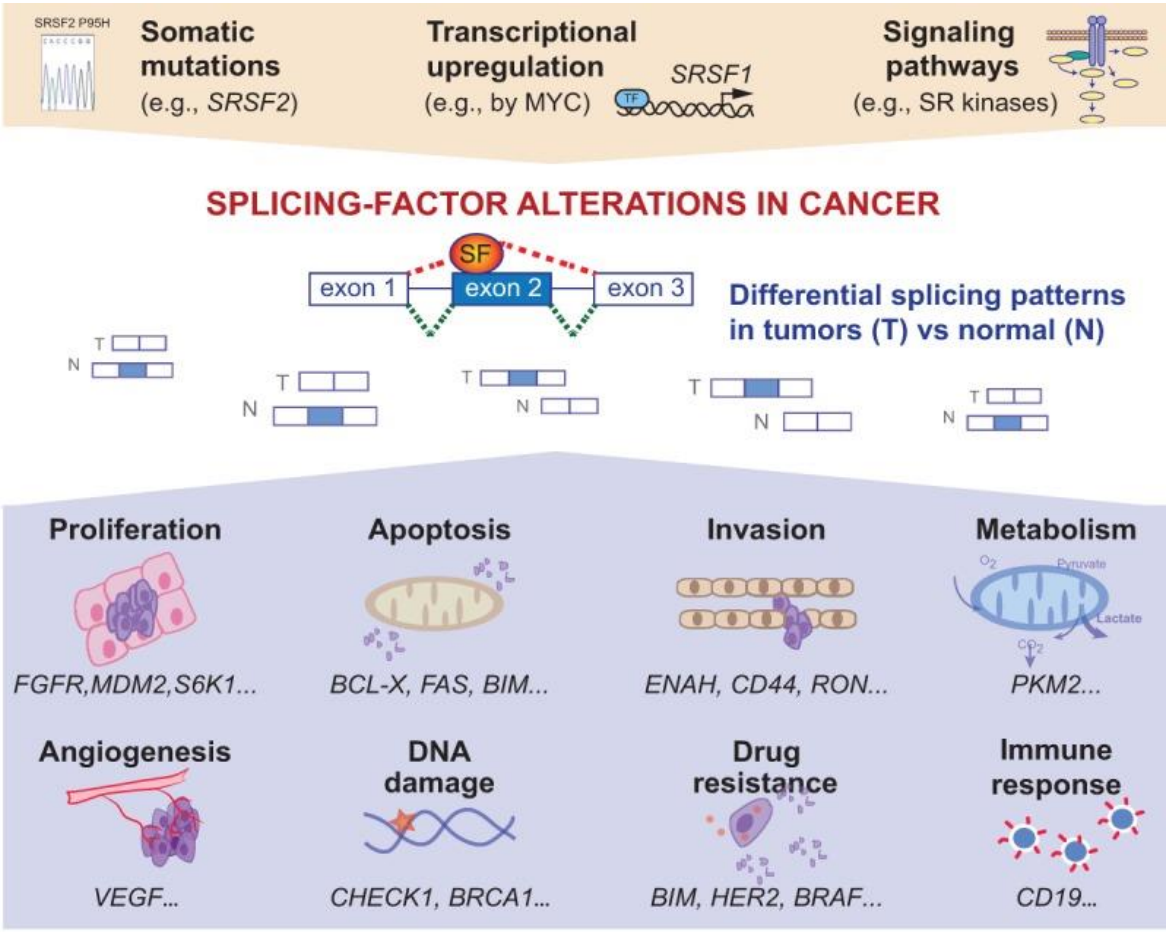

Figure 2. The role of SFs in cancer (25)

For the first time, the work shows that HCC tissues and cell lines upregulate RECQL4, and loss of RECQL4 function maintains HCC cell proliferation, migration, invasion, and EMT. Another study found a favorable connection between RECQL4 and SRSF1 in HCC tissue samples. SRSF1 is an RNA-

binding protein that stabilizes and promotes RECQL4 mRNA translation. Further tests on SRSF1 loss of function reveal that SRSF1 overexpression counteracts the inhibitory impact of RECQL4 silencing on HCC cell proliferation, migration, and invasion via binding to RECQL4 mRNA, altering HCC progression (41).

Raf-MEK-ERK pathway activation and cell transformation result from the downregulation of the dominant-negative isoform of A-Raf caused by the overexpression of hnRNP A2 in HCC (42). The study has elucidated the mechanism that generates mitotic insulin receptor isoform A (IR-A) and has found a novel link between the IR and EGFR pathways in HCC. The upregulation of IR-A during the progression of liver cell carcinogenesis might contribute to the negative consequences of high insulin levels on HCC (43).

Copy number variations of somatic mutations are often seen in HCC and may serve as a characteristic trait of liver cancer progression. Variations in SF copy numbers are frequent in HCC and could be a typical characteristic of the development of liver cancer. Copy number

variations and methylation have a major impact on the intricate expression control of SFs. Significant mutation patterns are shown by several SFs (44). PRPF6 overexpression boosts liver cancer and estrogen receptor signaling. In HCC, PRPF6, an SF, enhances AR autotranscription and activates the oncogenic AR/AR-Vs activity (45). Alternative forms or variations of SFs may result in an aberrant arrangement of AS events, which in turn impacts the synthesis of proteins downstream (25). SFs, being significant regulators of AS events, are vital in determining the development and progression of HCC. Mutations in SF genes are generally mutually exclusive (24).

SFS AND HCC DIAGNOSIS, TREATMENT, AND PROGNOSIS

BANF1, PLOD3, and SF3B4 molecular markers reveal early HCC in precancerous lesions and suggest liver cancer drivers (46). SF3B4, an SF derived from extracellular vesicles, serves as a non-invasive diagnostic biomarker for early HCC (47). The Ras signaling pathway promotes mouse HCC via the SRSF3-regulated coiled-coil domain with 50 splicing variants. Research indicates that the HBx/SRSF3/14-3-3β complex controls CCDC50S overexpression and accelerates HCC development via the Ras/Foxo4 signaling pathway. These findings imply that CCDC50S may be a viable HCC therapeutic target and a diagnostic and prognostic biomarker (48). SRSF1 is essential for HCC growth and development. Therefore, targeting SRSF1 may be a potential treatment. ERK may increase cell proliferation, survival, and IL-6 production to cause HCC. SF2 deletion somewhat increases TNF-α-induced cell death and partly suppresses IL-6 production since it stimulates ERK activation in these cells. Current evidence suggests SF2 may treat HCC (49).

The developed prognostic model identifies DNAJC6 and IGF2BP3 as risk factors, and ZC3H13 and DDX19B as protective factors. Previous research has shown a significant increase in the expression of DNAJC6 in HCC. This increase is strongly linked to the advancement of tumors and a negative prognosis for patients with HCC (50). This could be because DNAJC6 encourages the activation of the TGF-β pathway, which in turn leads to EMT and boosts the proliferation and invasion of HCC cells (51).

HCC prognosis depends on certain SFs. HCC tissues upregulate cancer-embryonic SF MBNL3, which promotes lncRNA-PXN-AS1 exon 4 inclusion. The transcript of lncRNA-PXN-AS1, including exon 4, may boost PXN mRNA expression by binding to its 3' UTR and shielding it from miR-24 degradation, enhancing HCC carcinogenesis and suggesting a poor prognosis (52). SF3B1 is a constituent of the central splicing complex and is upregulated in HCC. Increased expression of SF3B1 modifies the way KLF6 is spliced, and this alteration is strongly linked to a negative prognosis in individuals with HCC (32). The significance of SFs' aberrant overall expression in HCC, their prognostic relevance, and the biological activities they relate to cannot be overstated.

INSR, a target gene, shows a favorable correlation with tumor recurrence risk in human HCC when expressed as an AS subtype. Higher SF expression levels are associated with longer overall survival times (37). SRSF2 has significant levels of expression in HCC, and its expression levels correlate positively with tumor differentiation and TNM staging. It is linked to the spread of cancer cells to lymph nodes and other parts of the body, and is directly tied to the levels of alpha-fetoprotein in the blood. Additionally, it has an impact on the length of life after surgery in patients with HCC (53).

EXISTING PROBLEMS AND CHALLENGES

Despite advancements in the investigation of SFs in HCC, certain obstacles and inquiries remain unresolved. To begin with, the exact mechanisms by which SFs function in HCC are not yet fully comprehended. Concerning the relationship between particular SFs and the development and occurrence of HCC, there is a dearth of comprehensive research. Additional investigation is required to elucidate the precise functions of SFs in the progression of liver cancer.

Secondly, the diversity and complexity of SFs pose challenges for research. With numerous types of SFs and intricate regulatory interactions, investigating their functions and mechanisms becomes a significant undertaking. Systematically analyzing the roles of different SFs in HCC remains a critical topic in current research.

Additionally, addressing individual variability among HCC patients presents another hurdle. The mechanisms underlying HCC occurrence and progression may differ significantly between patients. Considering that SFs play a crucial regulatory role, their expression patterns and regulatory modes may vary substantially among patients, emphasizing the need for personalized treatment approaches.

In summary, while progress has been made in understanding SFs in HCC, several challenges persist. Future research should delve deeper into the mechanisms of SFs, address their diversity and complexity, and consider individual differences among liver cancer patients to advance this field.

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